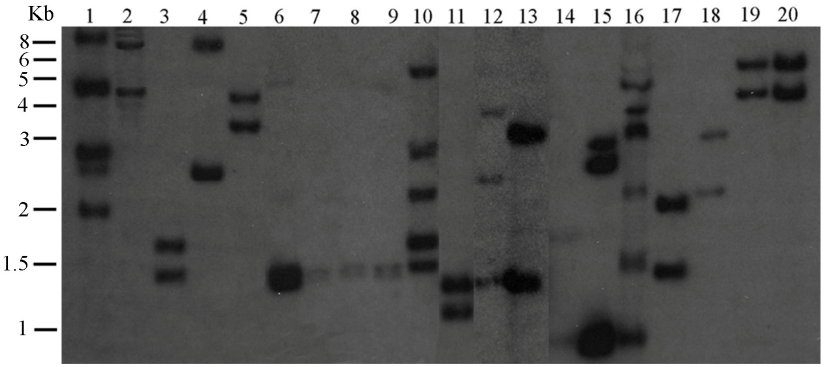
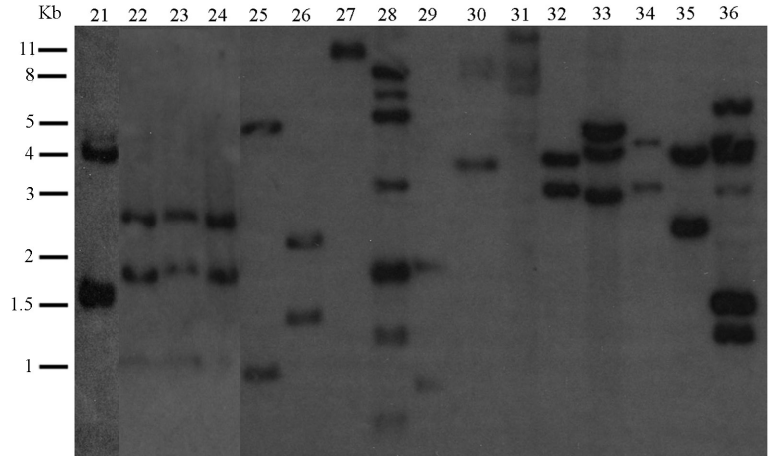
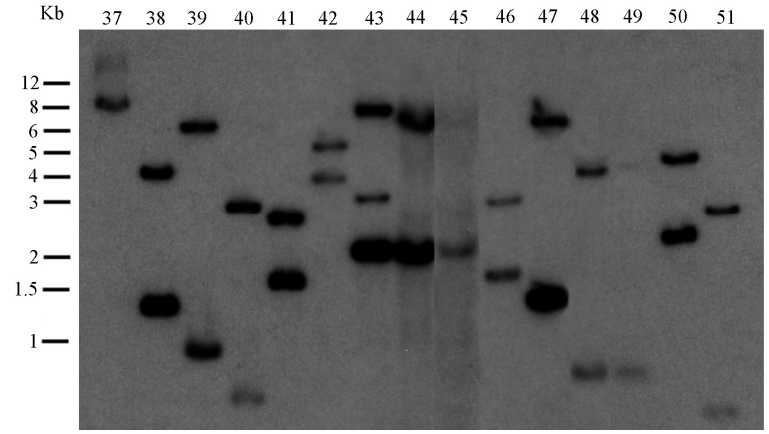
SOUTHERN 1				
Lane	**Mutants**	**Size (bp)**	**Size (bp)**	**Comments**
1 - 2	*bot1*Δ	4890	8645	Obtained the bands of the expected size in well #2. This mutant was used for further experiments
3	*byr4*Δ	1599	1557	Obtained the bands of the expected size
4	*bur6*Δ	2622	8260	Obtained the bands of the expected size
5	*cdc14*Δ	3740	4835	Obtained the bands of the expected size
6 - 10	*cet1*Δ	3382	1353	Only the lower band is correct in # 6 - 9. The mutant corresponding to well #6 was used for further experiments
11	*erg8*Δ	1264	1049	Obtained the bands of the expected size
12 - 13	*fba1*Δ	8192	1266	Mutant corresponding to well #13 has two hybridization bands, although only the lower is of the expected size. This mutant was used for further experiments
14	*fol1*Δ	1720	849	Obtained the bands of the expected size
15 - 16	*hrb1*Δ	3293	816	Obtained the bands of the expected size in #15 where there is also one additional hybridization band. The mutant corresponding to well #15 was used for further experiments
17	*kei1*Δ	1316	2051	Obtained the bands of the expected size
18	*ktr3*Δ	3682	2115	Obtained the bands of the expected size
19 - 20	*mdm10*Δ	5999	4572	Obtained the bands of the expected size in both candidate transformants, and the one corresponding to well #19 was used for further experiments



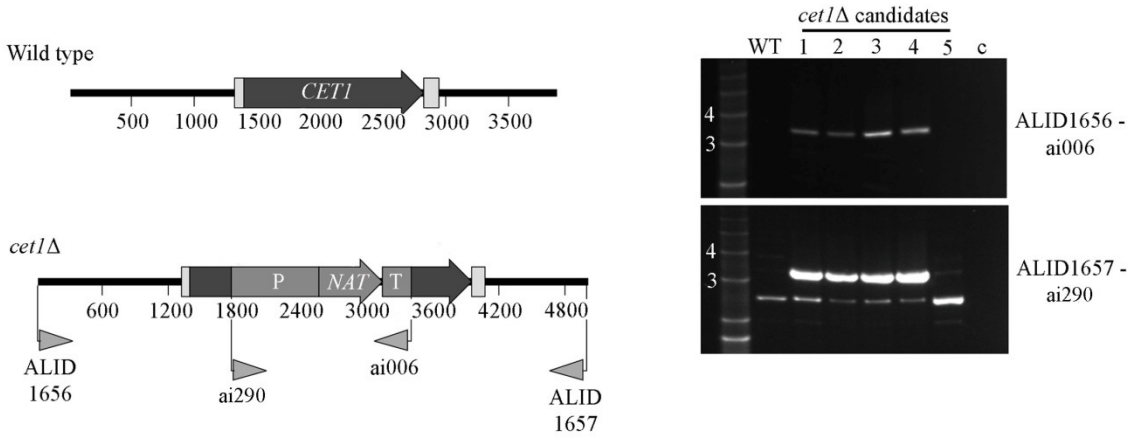
SOUTHERN 2				
Lane	**Mutants**	**Size (bp)**	**Size (bp)**	**Comments**
21	*mdm34*Δ	1605	4737	Obtained the bands of the expected size
22 - 24	*mgm101*Δ	2617	1880	Obtained the bands of the expected size in all the three *mgm101*Δ candidate mutants selected. The mutant corresponding to well #23 was used for further experiments
25	*mmm1*Δ	4919	999	Obtained the bands of the expected size
26	*mrpl7*Δ	2146	1381	Obtained the bands of the expected size
27	*mrpl31*Δ	11207	12706	Obtained the bands of the expected size
28	*mrps18*Δ	6672	17142	There are multiple hybridization bands. This mutant was used for further experiments
29	*mvd1*Δ	2096	883	Obtained the bands of the expected size
30 - 31	*nam9*Δ	7856	9211	Obtained the bands of the expected size in #31. This mutant was used for further experiments
32	*pwp1*Δ	2933	3674	Obtained the bands of the expected size
33- 34	*pdc1*Δ	2571	4039	Both mutants do not have the bands of expected size, even though the PCR is positive (see below). The mutant in well #33 was used both for genetic analysis and haploinsufficiency, while the mutant in well #34 was only used for the haploinsufficiency experiment as it is unable to produce spores for genetic analysis
35	*rib2*Δ	3867	2289	Obtained the bands of the expected size
36	*rib3*Δ	2292	3768	There are multiple hybridization bands. This mutant was used for further experiments



SOUTHERN 3				
Lane	**Mutants**	**Size (bp)**	**Size (bp)**	**Comments**
37	*rsa4*Δ	15235	8523	Obtained the bands of the expected size
38	*rsc9*Δ	1382	4221	Obtained the bands of the expected size
39	*rsm18*Δ	6730	945	Obtained the bands of the expected size
40	*saf2*Δ	2995	592	Obtained the bands of the expected size
41	*sec5*Δ	1660	2743	Obtained the bands of the expected size
42	*sen54*Δ	3877	5331	Obtained the bands of the expected size
43 - 45	*sfi1*Δ	2103	7811	Obtained the bands of the expected size in #44 and #45; the mutant corresponding to well #44 was used for further experiments
46	*CNAG_ 00592*Δ	1713	3036	Obtained the bands of the expected size
47	*thp1*Δ	5456	1451	Obtained the bands of the expected size
48 - 49	*tim54*Δ	3901	279	Same hybridization pattern for both *tim54*Δ candidates; only the higher band is of the expected size. Both mutants were used for further experiments.
50	*trl1*Δ	4390	2277	Obtained the bands of the expected size
51	*trr1*Δ	2703	564	Obtained the bands of the expected size

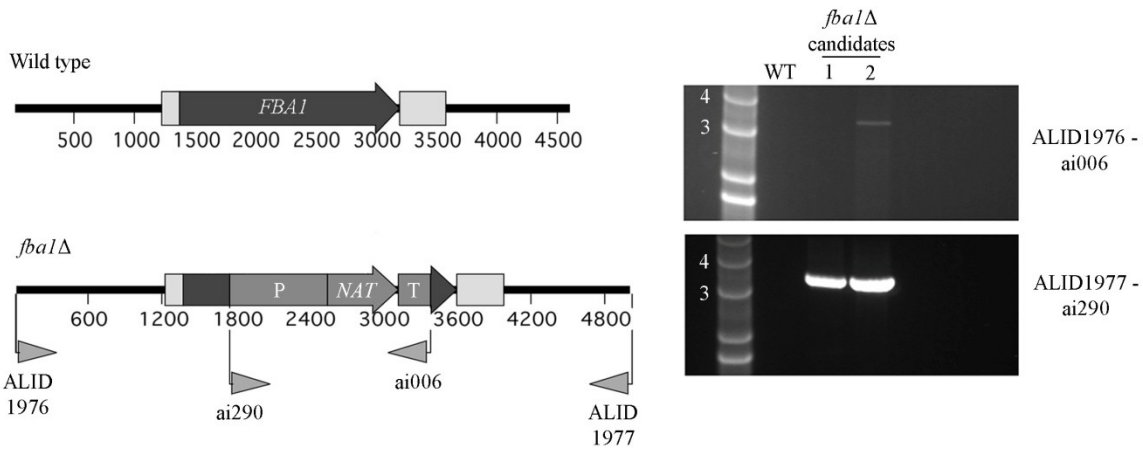


A) Gene replacement and PCR confirmation for the gene *CET1*



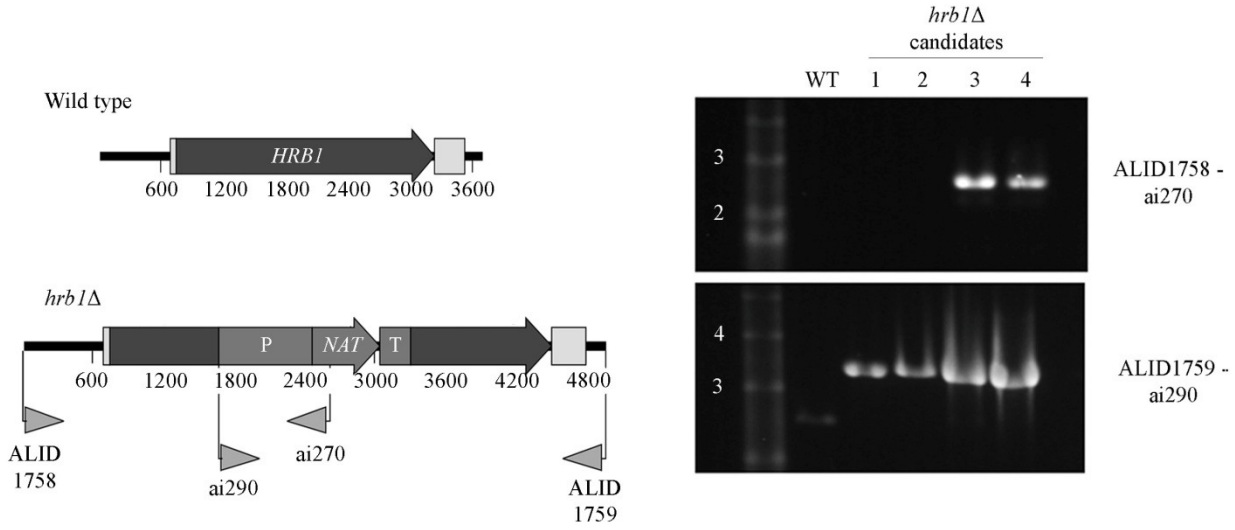
Comment: *cet1*Δ candidates #1 - 5 correspond to wells #6 - 10 of the Southern blot 1. Candidates *cet1*Δ #1, #2, #3 and #4 have the same amplification pattern, and *cet1*Δ #1 (#6 of Southern 1) was used for further experiments. PCR with primers ALID1657-ai290 produced a non-specific amplicon of ~2.5 Kb. c: water control.

B) Gene replacement and PCR confirmation for the gene *FBA1*



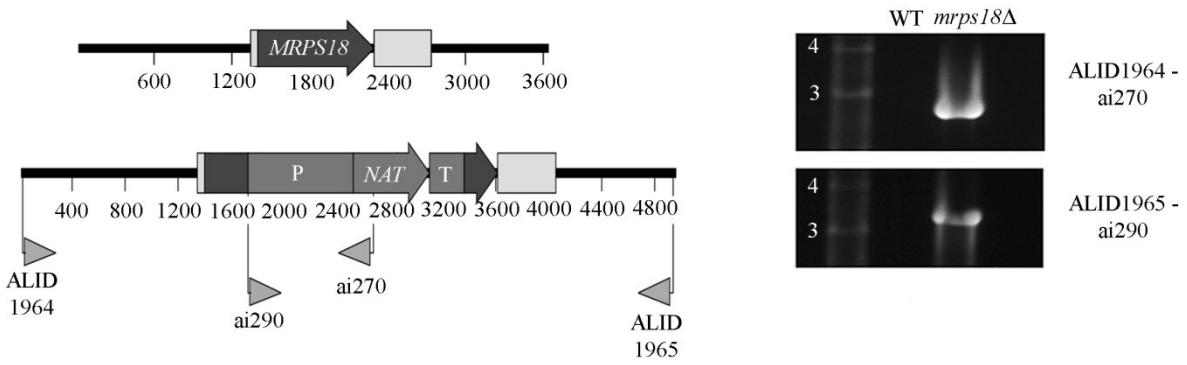
Comment: *fba1*Δ candidates #1 and #2 correspond to wells #12 and #13 of the Southern blot 1. PCR for candidates *fba1*Δ #2 showed correct integration of the gene replacement construct, while in candidate *fba1*Δ #1 most likely only the right side is correctly integrated. Therefore, mutant *fba1*Δ #2 (#13 of Southern 1) was used for further experiments.

C) Gene replacement and PCR confirmation for the gene *HRB1*



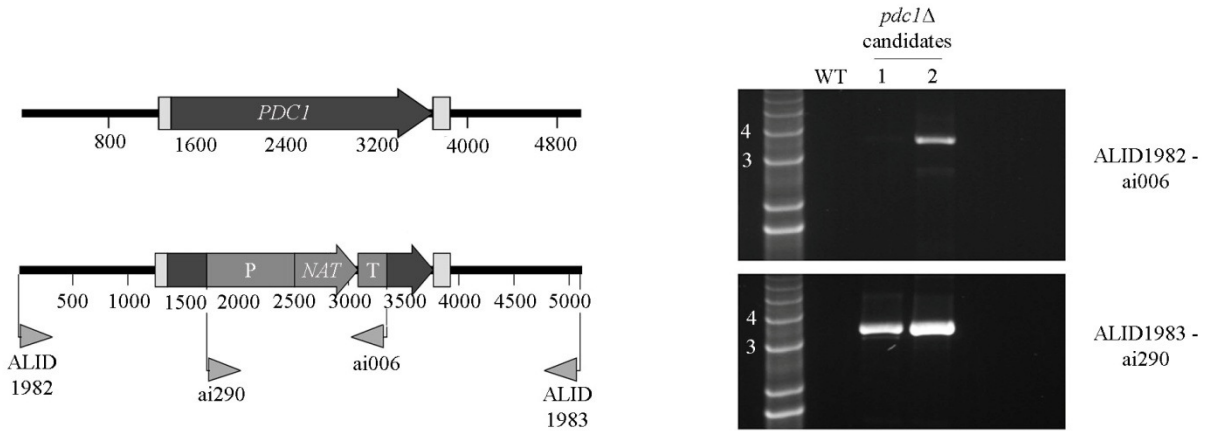
Comment: PCR for candidates *hrb1*Δ #1 and #2 shows that probably only the right side of the gene replacement construct is correctly integrated, while for candidates *hrb1*Δ #3 and #4 both left and right sides are correctly integrated. *hrb1*Δ candidates #3 and #4 correspond to well #15 and #16 of the Southern blot 1. Mutant *hrb1*Δ #3 (#15 of Southern 1) was used for further experiments.

D) Gene replacement and PCR confirmation for the gene *MRPS18*



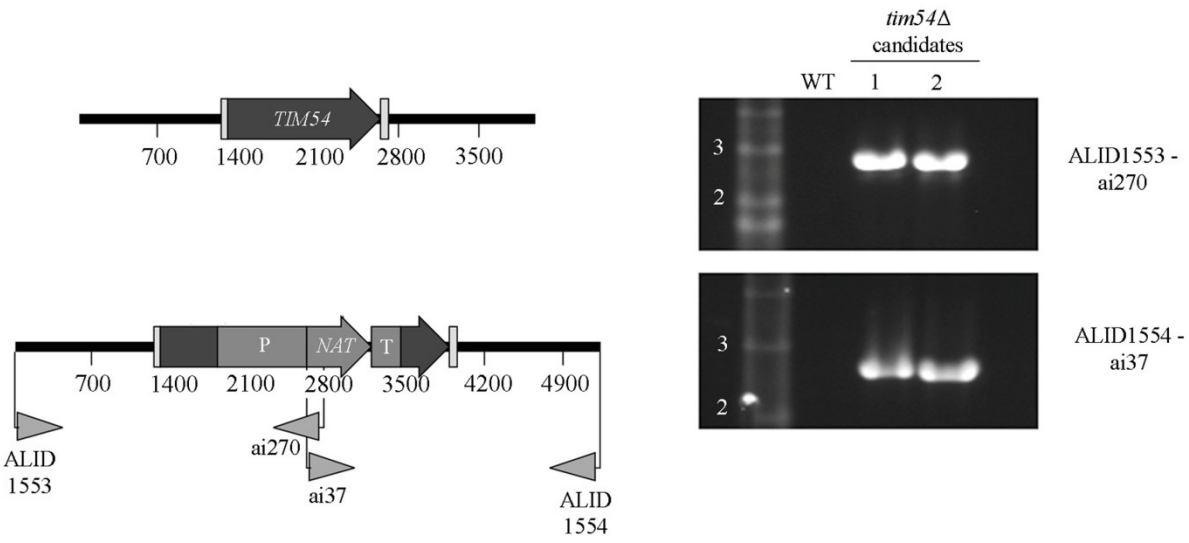
Comment: one candidate *mrps18*Δ strain was isolated (#28 of Southern 2). The PCR produced bands of the expected size, and this mutant was used for further experiments.

E) Gene replacement and PCR confirmation for the gene *PDC1*



Comment: *pdc1*Δ candidates #1 and #2 correspond to wells #33 and #34 of the Southern blot 2; PCR showed correct integration of the gene replacement construct, even though for candidate *pdc1*Δ#1 the amplicon obtained using primers ALID1982-ai006 was weak. The mutant *pdc1*Δ#1 was used both for genetic analysis and haploinsufficiency, while the mutant *pdc1*Δ#2 was only used for the haploinsufficiency experiments as the strain was unable to produce spores for genetic analysis.

F) Gene replacement and PCR confirmation for the gene *TIM54*



Comment: *tim54*Δ candidates #1 and #2 correspond to wells #48 and #49 of the Southern blot 3; PCR showed correct integration of the gene replacement construct for both candidates, which were both used for further experiments.